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I/LA30-A

Immunoassay Interference by Endogenous Antibodies; Approved Guideline

This guideline discusses the nature and causes of interfering antibodies, as well as their effects on immunoassays and mechanisms by which interference occurs. Methods to identify and characterize the interferences are addressed along with assessment of methods used to eliminate interference.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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For additional information on committee participation or to submit comments, contact CLSI.

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Immunoassay Interference by Endogenous Antibodies; Approved Guideline

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Abstract

Clinical and Laboratory Standards Institute document I/LA30-A—*Immunoassay Interference by Endogenous Antibodies; Approved Guideline* presents information on the origin, nature, and prevalence of circulating endogenous antibodies, which cause interference with immunoassay results. The mechanisms of the interference along with some specific examples are included. To address the problem, recommendations for regulatory bodies, reagent manufacturers, and laboratorians are provided.

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Contents

Foreword

This guideline describes methods for identification and potential elimination of immunoassay interference caused by antibodies in patient specimens. These circulating endogenous antibodies can cause falsely increased or decreased results for analytes measured by immunoassay. Interferences are assay-dependent and often go unrecognized, thus leading to misinterpretation of results. When results falsely signify an underlying medical condition, unnecessary follow-up testing or treatment can occur. Assay interferences also can cause failure to recognize disease. Even though in the design and development of immunoassays, the issue of interfering antibodies has been addressed, complete elimination of interference has not been possible. Clinicians thus need to be aware of the limitations of immunoassays. Test results that are inconsistent with other sources of medical information and do not fit the clinical picture should be considered suspect. This requires awareness of this type of problem and good communication for both laboratory personnel and the patient's physician.

Key Words

Antianimal antibodies, autoantibodies, endogenous antibodies, heterophile antibodies, immunoassay, interference

Immunoassay Interference by Endogenous Antibodies; Approved Guideline

1 Scope

This guideline discusses the nature and causes of interfering antibodies as well as their effects on immunoassays and mechanisms by which interference occurs. Methods to identify and characterize the interferences are addressed along with assessment of methods used to eliminate interference. This document suggests guidelines for regulatory bodies, manufacturers, and laboratorians in their roles identifying and eliminating endogenous interfering antibodies in patient specimens. Although examples of specific assay interferences are included, the document does not intend to describe all methods or analytes where antibody interference has been reported. The guideline does not address other types of immunoassay interferences, such as hemolysis, cross-reacting substances, and drug interference, except when the drug is an antibody. The intended users of the guideline are organizations responsible for regulatory oversight of immunoassay reagent production, manufacturers of immunoassay reagents, and laboratorians performing immunoassays.

2 Introduction

Because of their sensitivity and specificity, immunoassays are important diagnostic tools allowing measurement of a wide variety of analytes. Immunoassays, however, are subject to a number of interferences including those caused by circulating endogenous antibodies. Interference can occur because of heterophile antibodies, antianimal antibodies, or autoantibodies. The interfering antibodies can give rise to falsely high or, less commonly, falsely low results. The erroneous result is recognized as being inconsistent with the patient's clinical picture, but often it is clinically difficult or impossible to recognize an assay result as spurious. Additionally, it may be difficult to ascertain by commonly used laboratory procedures that a given result is erroneous. The laboratory procedures generally used to identify the presence of interfering antibodies are demonstration of a nonlinear response to dilutions, addition of nonimmunoglobulin protein to block the interfering antibody, or use of an alternate immunoassay. None of these commonly used procedures, however, can identify interference reliably in all cases. The magnitude of the problem of antibody interference is unknown with certainty, because wide variation in prevalence has been described depending on the detection methods used and the populations studied. Circulating endogenous antibodies may arise from incidental or occupational exposure to foreign protein, use of antibodies as diagnostic or therapeutic agents, following infection or vaccination, or for unknown reasons. The interference is variable, complex, and unpredictable because of the wide range of affinities and avidities found among the various endogenous antibodies that can be encountered. The antibodies may react with the analyte, the reagent antibodies, or both. There are also reports of antibodies interfering with the immunoassay detection systems. Interfering antibodies are not only difficult to recognize but are problematic to eliminate. Nonlinear response to dilutions cannot always be identified in the presence of interfering antibodies. The interfering antibodies can have high titer or avidity and thus, it may be difficult to eliminate the interference with blocking agents. Interfering antibodies may react with various types of assay antibodies and thus may interfere in different assay types. The intent of this document is to increase awareness of the problem of interfering antibodies and to suggest approaches to minimize their impact on patient care. The intent of the subcommittee is to repeat pertinent information under various subheadings in the document.

3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major feature of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all infectious agents and thus are

more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.[1] For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to CLSI document M29.[2]

4 Terminology

4.1 Definitions

affinity – the force of attraction between molecules.

antianimal antibodies – antibodies that show strong avidity for test antibodies of one species, but the antibody may cross-react with immunoglobulins from other species.

antibody – a substance formed in the body in response to a foreign protein (an antigen) that interacts only with that substance; however, it may also bind to structurally related substances.

avidity – net affinity of all binding sites of antibodies.

cryoglobulin – a mixture of globulins that precipitates when cooled and dissolves when reheated to body temperature.

heterophile antibodies – antibodies produced against poorly defined antigens that react with immunoglobulins from two or more species.

rheumatoid factors – antibodies that bind to the constant or Fc portion of other immunoglobulins.

secondary antibody – an antibody that recognizes and binds a primary antibody.

4.2 Acronyms/Abbreviations

Ab	(Ag-specific) antibody
AFP	alphafetoprotein
Ag	antigen
ALG	antilymphocyte globulin
CB	competitive protein binding
CK-MB	creatine kinase MB isoenzyme
CLIA	chemiluminescent immunoassay
CRP	C-reactive proteins
EBV	Epstein-Barr virus
ELISA	Enzyme-Linked Immunosorbent Assay
FIA	fluoroimmunoassay
FPIA	fluorescence polarization immunoassay
FSH	follicle stimulating hormone
HAAA	human antianimal antibodies
HAMA	human antimouse antibodies
HARA	human antirabbit antibody
HBV	hepatitis B virus
hCG	human chorionic gonadotropin
IgG	immunoglobulin G
IgM	immunoglobulin M
LH	luteinizing hormone

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care.* The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessments—External & Internal	Facilities & Safety

I/LA30-A addresses the QSEs indicated by an "X." For a description of the other document listed in the grid, please refer to the Related CLSI Reference Material section below.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External & Internal	Process Improvement	Customer Service	Facilities & Safety
					X M29						M29

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*.

Related CLSI Reference Material*

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

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